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## Seaweed cultivation pilot trials – towards culture systems and marketable products

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### Abstract

Globally, seaweed is the largest aquaculture production by volume at over eight million wet metric tonnes per annum (FAO 2003). Mostly this production is for traditional foods in Asia and the commodity markets of agar, alginates and carrageenans. However, there is also untapped potential in smaller, high product value markets for nutritional and health applications. This is where Australia's best investment in a seaweed industry may lie.

Australia has a number of advantages and opportunities that present themselves with regard to the development of a seaweed cultivation industry. Of particular advantage for Australia is the large coastal zone area with unpolluted waters. This fits very well with the production of high quality health and food products that require internationally recognised traceability and testable safety standards. The development of seaweed cultivation technology in the coastal zone could also pave the way for new crops in large areas of saline affected agricultural land; an as yet unrealised ambition. Alternative saline tolerant and low freshwater demanding crops will be important to food and water security in a changing climate. The expansion of land-based aquaculture industries in Australia also present an opportunity to investigate the development of seaweed cultivation technology by making use of aquaculture infrastructure, such as seawater intakes, to develop scaled-up cultivation systems. This also provides environmental benefits to the aquaculture industry.

There are however serious challenges to overcome. Australia has no tradition in the cultivation of seaweed and application of the science supporting it. The propagation and control of complex biological lifecycles and the physiological requirements of Australian seaweeds are not well established. As for any new and emerging industry, lessons need to be learned from the overseas experience and new and innovative solutions for the Australian context need to be developed. In addition, Australia will have to develop its own track record, profile and niche products in this industry where the greatest value is likely to come from products with high nutritional and health benefits.

This report presents findings that demonstrate an untapped potential for cultivation of a number of local Australian seaweed species, but it also identifies the challenges facing commercial-scale production. Importantly, it also provides evidence that Australia has the capacity and potential to undertake cutting edge screening and development of healthy seaweed products, in particular, products with nutraceutical and anti-cancer applications.

This report is an addition to RIRDC's diverse range of over 2000 research publications and it forms part of our New Plant Products R&D program, which aims to facilitate the development of new industries based on plants or plant products that have commercial potential for Australia.

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# Seaweed Cultivation Pilot Trials

— *Towards culture systems and marketable products* —

RIRDC Publication No. 10/184



RIRDC Innovation for rural Australia





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Development Corporation**

# **Seaweed Cultivation Pilot Trials**

**Towards culture systems and marketable products**

By Dr Pia Winberg, Dr Danielle Skropeta & Ms Alex Ullrich

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# Foreword

Globally, seaweed is the largest aquaculture production by volume at over eight million wet metric tonnes per annum (FAO 2003). Mostly this production is for traditional foods in Asia and the commodity markets of agar, alginates and carageenans. However, there is also untapped potential in smaller, high product value markets for nutritional and health applications. This is where Australia's best investment in a seaweed industry may lie.

Australia has a number of advantages and opportunities that present themselves with regard to the development of a seaweed cultivation industry. Of particular advantage for Australia is the large coastal zone area with unpolluted waters. This fits very well with the production of high quality health and food products that require internationally recognised traceability and testable safety standards. The development of seaweed cultivation technology in the coastal zone could also pave the way for new crops in large areas of saline affected agricultural land; an as yet unrealised ambition. Alternative saline tolerant and low freshwater demanding crops will be important to food and water security in a changing climate. The expansion of land-based aquaculture industries in Australia also present an opportunity to investigate the development of seaweed cultivation technology by making use of aquaculture infrastructure, such as seawater intakes, to develop scaled-up cultivation systems. This also provides environmental benefits to the aquaculture industry.

There are however serious challenges to overcome. Australia has no tradition in the cultivation of seaweed and application of the science supporting it. The propagation and control of complex biological lifecycles and the physiological requirements of Australian seaweeds are not well established. As for any new and emerging industry, lessons need to be learned from the overseas experience and new and innovative solutions for the Australian context need to be developed. In addition, Australia will have to develop its own track record, profile and niche products in this industry where the greatest value is likely to come from products with high nutritional and health benefits.

This report presents findings that demonstrate an untapped potential for cultivation of a number of local Australian seaweed species, but it also identifies the challenges facing commercial-scale production. Importantly, it also provides evidence that Australia has the capacity and potential to undertake cutting edge screening and development of healthy seaweed products, in particular, products with nutraceutical and anti-cancer applications.

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**Craig Burns**

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Dr. Danielle Skropeta is a research chemist and senior lecturer at the University of Wollongong. Danielle's research has a strong focus on bioactive compounds from marine sources.

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# Abbreviations and definitions

IMTA	Integrated Multi-Trophic Aquaculture
Seaweed	marine macroalgae



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# Executive Summary

## What the report is about

This project builds on interest in the potential for seaweed cultivation in Australia, by contributing to knowledge about a range of local Australian seaweed species and examining their cultivation and health potential. Since the 1990's, there has been a slow but continued interest in pursuing the potential for seaweed industries and markets in Australia (Lee 2007, Lee and Momdjian 1997) including inland cultivation trials (Cordover 2007) and more recently reviews on the applications of seaweed as an important nutritional component of the Australian diet (Winberg et al. 2009). However progress towards realising a viable seaweed industry relies on identifying the most immediate opportunities for viable cultivation technology and markets that take advantage of existing knowledge, infrastructure and developing vertically integrated industry chains.

Of particular interest is the application of seaweed cultivation technology in Integrated Multi-Trophic Aquaculture systems through improved production efficiencies, reduced environmental impacts and diversification of products for producers. This project established the suitability of 18 species of local seaweeds to culture conditions at laboratory and pilot commercial scales. Twelve taxa were also screened for anti-cancer activity.

## Who is the report targeted at?

The report is targeted at government agencies, aquaculture industry representatives and aquaculture producers, food and nutritional companies and researchers to demonstrate the untapped potential of seaweed as a saline crop in Australia. Australia is new in the field of seaweed cultivation and product development. The hurdles that need to be addressed to realise a fully vertically integrated industry within Australia requires learning from overseas experiences and research and development towards application in the Australian context.

The general public also needs to be educated about the potential health benefits of seaweed species in order to demonstrate and build acceptance and a market demand. Therefore this document is targeted at an educated and nutritionally interested general Australian public as well.

## Background

With a systematic approach to achieving a viable seaweed industry for Australia in mind, Winberg et al. (2009) recommended that Integrated Multi-Trophic Aquaculture (IMTA) systems provide an opportunity for Australia to develop a seaweed cultivation industry and markets. The use of seaweeds as a biofilter for effluent was first suggested in the 70's by Ryther et al. (1975) and since then only a handful of macroalgal species have been fully investigated for integration into aquaculture. In Australia, the nitrogen and phosphate removal efficiency of *Gracilaria edulis* has been investigated by Jones (2001), and in tropical climates *Caulerpa* (Nicholas and de Nys 2008) and other green macroalgal species (de Paula Silva et al. 2008b) have been trialled, but this has barely scratched the surface of possibilities for seaweed culture in Australia.

To speed up the development of the industry, it is important that the potential health properties of these seaweeds are investigated while the cultivation of seaweeds is being trialled. The core opportunity for marketing of high value seaweed products is related to the many potential health benefits of seaweed compounds. Extracts from seaweeds contain a complex mixtures of hundreds of natural compounds, to which health benefits such as antioxidant, anti-cancer, anti-inflammatory, and immuno-stimulatory benefits have been ascribed (Winberg et al. 2009). Seaweeds are known to produce cytotoxic (cancer cell killing) compounds such as kinase inhibitors including stypodiquinonic acid from the brown alga *Stypodium zonale* (Wessels et al. 1999), cyloartanol sulfates from *Tydemania Expeditionis* (Govindan 2008), and sulfated triterpenoids from a green alga belonging to the *Tuomoya*

genus (Clement 2003). This project conducted assays on extracts from 12 seaweed taxa to determine if there was cyto-toxic activity. Therefore screening for kinase inhibition properties of seaweed extracts selected in this project was selected as priority method for assessing potential anti-cancer properties.

From a practical perspective, the development of seaweed cultivation technology utilises infrastructure such as seawater intake systems that are already in place for other purposes, thus reducing the risk of investment in trialling and scaling up cultivation systems. In addition there are environmental and economic opportunities as seaweed cultivation in IMTA can provide improved environmental outcomes, and take advantage of valuable nutrient resources that are otherwise considered a pollutant and waste. Seaweed also has the potential to be integrated into the aquaculture industry markets such as abalone feed or as an ingredient in nutritionally enhanced fish foods. Research in other countries has shown that seaweed integration with other aquaculture is technically feasible, can be economically viable and makes environmental sense (Bolton et al. 2009, Neori et al. 2004).

## **Aims/objectives**

The objectives of this research project were to set up pilot tumble culture trials, at both a laboratory and pilot farm scales, for a range of seaweed species native to the NSW south coast. In addition, a selection of these were screened for anti-cancer activity.

The outcomes from this study will help build an Australian seaweed industry by identifying the opportunities and potential for seaweed, and also the gaps in our knowledge that need to be addressed.

## **Methods used**

### *Seaweed selection*

The physiological requirements, the biological cycles and propagation of different seaweed species can vary widely. In addition, only a few seaweeds will be considered suitable for integration into IMTA systems with high nutrient loads and tumble culture conditions. Therefore, the species considered here for cultivation and cancer screening trials were selected on the basis of some or all of the following criteria:

- abundant and native to the NSW south coast
- reported high nutrient stripping capacity from other studies
- reported elsewhere as cultivable, and in particular in integrated fish or shellfish culture systems
- medical, nutritional or other high value marketable product potential
- opportunistic species that developed within cultivation trials

### *Laboratory cultivation*

Tumble culture was chosen for these trials. It is considered to be one of the less-labour-intensive seaweed cultivation technologies. Tumble culture experiments were set up in the laboratory in artificially lit aquaria. Artificial fertilizers and natural seawater were used in the system that would determine if the different seaweed species could adapt to artificial tumble culture conditions easily. Specific experiments were also undertaken to determine the nutrient uptake rates of three seaweed species, the effects of light on growth and chlorophyll content, and also whether standard aquaculture water sterilisation methods had a negative impact on some seaweeds.

### *Pilot commercial cultivation*

The species of most marketable and reputable potential in IMTA systems were trialled in a commercial pilot scale IMTA system with fed marine fish and >1m<sup>3</sup> tumble culture modules.

### *Anti-cancer screening*

One way to determine if and how an extract can kill cancer cells is to measure inhibitors of kinase enzymes which are crucial to the survival of cancer cells. Kinase inhibitors provide a new target for anticancer agents that are more specific, efficacious and with less toxic side effects, and there are several examples already in clinical trials (Dancey and Sausville 2003). This project conducted assays on extracts from 12 seaweed taxa to determine if there was cyto-toxic activity expressed as kinase-inhibition.

### **Results/key findings**

There were 18 taxa of seaweeds that were collected and screened for cultivation trials and/or anti-cancer activity. The criteria used to select these species are provided in table 1.

**Table 1. Criteria used and the and selected species for tumble culture and/or trials anti-cancer screening in this project.**

Species	abundant in NSW	nutrient stripping	cultivable	medical / nutritional value	opportunistic
"Brown ribbon" weed	yes				
<i>Codium</i> sp.	yes			yes	
<i>Colpomenia</i> sp. 1	yes				
<i>Dictyota</i> sp. 1	yes		yes		
<i>Ecklonia radiata</i>	yes			yes	
<i>Pterocladia</i> sp.	yes	yes	yes		
<i>Gelidium</i> sp.	yes		yes		
<i>Petalonia</i> sp.	yes		yes	yes	
<i>Phyllospora comosa</i>	yes				
<i>Porphyra</i> sp. 1	yes	yes	yes	yes	
<i>Porphyra</i> sp. 2	yes	yes	yes	yes	
<i>Sargassum</i> sp.	yes		yes	yes	
<i>Gracilaria</i> sp. "Spaghetti"	yes		yes		
<i>Ulva</i> sp. ( <i>Enteromorpha</i> and blade forms)	yes	yes	yes	yes	
Gelatinous reds			yes		yes
<i>Ceramium</i> sp.					yes
<i>Cladophora</i> sp.		yes			yes
<i>Bryopsis</i> sp.					yes

### *Pilot cultivation trials in laboratory conditions*

All red and green species of seaweeds showed good adaptation to cultivation in tumble culture conditions in the laboratory, however only *Ulva* sp., *Porphyra* sp., and some opportunistic species maintained good condition and had reasonable growth rates. *Ulva* and *Porphyra* require further investigation to develop propagation and grow-out protocols that reliably produce high yields and quality product, while mono-culture trials and the nutrient stripping capacity of the opportunistic species need to be determined. Brown seaweeds may prove difficult to cultivate in tumble culture conditions on their adult form.

### *Pilot commercial cultivation in IMTA*

Integration of seaweed as a biofilter component of a recirculation IMTA system was successful in terms of nutrient stripping, seaweed yield and fish health.

### Anti-cancer screening

Ethanol extracts of twelve species collected during 2008, were screened for in vitro inhibitory activity against protein kinase A, a key enzyme implicated in a range of diseases including cancer. Ten of the twelve samples were found to inhibit protein kinase A to some degree. Importantly, three species (2 browns (*Ecklonia* and *Sargassum* spp.) and 1 red (*Porphyra* spp.)), showed very high and replicable results for kinase inhibiting compounds.

### **Implications for relevant stakeholders for:**

The Australian primary industry sector needs to diversify and tackle new cultivation technologies that are aligned with a changing climate and reduction in seafood production. In particular the aquaculture industry requires increased efficiencies and environmental standards. Here we provide information on a range of seaweed species that hold potential for further development of high yielding cultivation technology, as well as high end marketable health properties, and in this way demonstrate the potential of seaweed as a new crop that can contribute to Australia's primary production. The information should provide support and justification for industry leaders to push for further development of seaweed cultivation technology in Australia.

Policy makers and primary industry bodies need to understand the full suite of potential future options for sustainable primary production in regional Australia. Global food shortages, seafood quality and the health benefits of seaweed and seafood demonstrate the opportunity for seaweed to play a key part in improving the sustainable primary production industry of regional Australia. Regional and coastal communities in particular are facing changes due to reduced productivity in the wild fishing sector, and also face challenges of developing new and sustainable industries, while long term applications of seaweed cultivation in inland saline affected areas can continue as a goal in the development of cultivation technology.

### **Recommendations**

This report demonstrates that there are numerous seaweed species in Australia that hold potential for a vertically integrated seaweed industry. This is not necessarily far off if we focus on the immediate opportunities of cultivation alongside existing aquaculture enterprises and target immediately-marketable products such as nutritional and healthy foods. However there are also clear barriers to seaweed cultivation attracting investment and becoming a commercial reality. Therefore, this document should provide some stimulus for pushing the tangible and short-term development in a seaweed industry for Australia.

Recommendations arising from this report that might provide the most immediately commercially viable seaweed cultivation opportunities include further refinement of cultivation and propagation protocols for species such as *Ulva* and *Porphyra*. In addition algal genetics research is required to confirm species identification and understand genetic expression of desirable properties. This would include the development of Australian product and streamlining the product through the process of safe food standards.

Companies in existing aquaculture enterprises should be supported in trying to adopt and scale up the technology of seaweed cultivation, particularly for land based facilities where efficiency and environmental gains are important.

Species that provide challenging in tumble culture conditions, i.e. *Ecklonia radiata* and *Sargassum* sp. still deserve further cultivation technology development as the potential health benefits could provide for substantially greater value even though this requires more complex cultivation systems. The application of health benefits also needs further investigation.

These concepts should be supported through R&D bodies and research organisations in collaboration with existing aquaculture industries that appreciate the opportunity for seaweed cultivation development.




# Introduction

## Context of project

This project builds on the interest in the potential for seaweed cultivation in Australia that started within the RIRDC portfolio in the 1990's. Since then, there has been a slow but continued interest in pursuing the potential for a seaweed industries in Australia (Lee and Momdjian 1997; Lee 2007) including inland cultivation trials (Cordover 2007) and more recently reviews on the applications of seaweed as an important nutritional component of the Australian diet (Winberg et al. 2009). However progress towards realising a viable seaweed industry relies on identifying the most immediate opportunities for viable cultivation technology and markets that take advantage of existing knowledge, infrastructure and vertically integrated industry chains. This report builds on this previous research to further progress the development of a seaweed cultivation industry in Australia (Table. 2).

**Table 2: Stages and current progress towards realising a commercially viable seaweed cultivation industry in Australia.**



	1) Identify Potential Markets and Health benefits	2) Testing cultivation technology	3) Vertical integration / development of markets	4) Commercial adoption of cultivation technology	5) Domestic supply to markets developed
Established	(Lee and Momdjian 1997)  (Lee 2007)  (Winberg et al. 2009)	(Cordover 2007)  (de Paula Silva et al. 2008)  (Nicholas and de Nys 2008)	Seaweed Australia industry body (under establishment)		Nutraceuticals (Marinova)  Fertilizers & supplements (King Island Produce)  (currently from imported, introduced or storm cast species)
	1990- 2009	2000	2009	Planning underway	Planning underway

This project adds to existing efforts and knowledge on Australian seaweed species with potential for cultivation and health benefits.

Trono (1989) stated that “the great successes in seaweed culture achieved in such countries as Japan and China are generally attributed to achievements in controlling the biological cycle and satisfying physiological requirements..... in the countries where these basic technologies are not yet available, the development of culture techniques in order to enhance production is the major concern”. This does not eliminate the potential for a seaweed industry in Australia, however it dictates that a carefully planned approach is needed to develop markets, attract investment, and take maximum advantage of what the Australian industry and environment have to offer. This is also supported by recommendations of previous inland saline water cultivation trials in Australia (Cordover 2007).

With a systematic approach to achieving a viable seaweed industry for Australia in mind, Winberg et al. (2009) demonstrated that Integrated Multi-Trophic Aquaculture (IMTA) systems provide an opportunity for Australia to develop a seaweed cultivation industry and markets. The development of seaweed cultivation technology can utilise existing infrastructure such as seawater intake systems that are in place for other purposes. Making use of such resources reduces the cost and risk of investment in the scaling up of cultivation technology. In addition there are environmental and economic opportunities as seaweed in IMTA can provide improved environmental standards of aquaculture and take advantage of valuable nutrient resources otherwise considered a pollutant and waste. In Australia, the nitrogen and phosphate removal efficiency of *Gracilaria edulis* has been investigated by Jones (2001), and in tropical climates *Caulerpa* (Nicholas and de Nys 2008) and other green macroalgal species (de Paula Silva et al. 2008) have been trialled with few other such investigations being carried out. Seaweed also has the potential to be integrated into the aquaculture industry markets such as abalone feed or as an ingredient in nutritionally developed fish foods. Research in other countries has shown that seaweed integration with other aquaculture is technically, economically and environmentally viable (Neori et al. 2004; Bolton et al. 2009).

## **How to start screening seaweeds for cultivation and market potential in Australia**

After establishing that IMTA systems represent a promising opportunity for seaweed cultivation, there are a number of important issues that need to be addressed to progress the industry further. In choosing species, those that have been proven as successful in cultivation systems elsewhere in the world and the technology used provides a good starting point for the development of Australian cultivation technology. In addition, the Australian coastline offers a multitude of endemic and other species (Sanderson 1997) that haven't been considered for cultivation, and field work and pilot trials could quickly identify species that appear to cope with tank cultivation conditions, high nutrient loads or that grow quickly and in abundance.

Seaweed selection for studies towards cultivation and marketable products in Australia should be considered upon two main criteria:

- demonstrated nutrient stripping capacity and/or the potential for IMTA culture, and
- relevant health potential of cultivated seaweeds to target the higher end market that will provide the best return on investment.

## **Nutrient stripping and cultivation potential**

The use of seaweeds as a biofilter for effluent was first suggested in the 70's by Ryther et al. (1975) and since then only a handful of macroalgal species have been fully investigated for integration into aquaculture (Table 3). In Australia, the nitrogen and phosphate removal efficiency of *Gracilaria edulis* has been investigated by Jones (2001), , and in tropical climates *Caulerpa* (Nicholas and de Nys 2008) and other green macroalgal species (de Paula Silva et al. 2008) have been trialled with few other such investigations being carried out. The nutrient uptake efficiencies for macroalgae that have been integrated in aquaculture are listed at Table 3 as an average reduction (%) in nutrient concentration from the influent water. Some studies focused macroalgal growth rates which can be used as a proxy for nutrient uptake efficiency, and therefore specific nutrient uptake rates are not provided for these taxa.

## Nutritional and health potential

While the cultivation of seaweeds needs to be a big focus of the R&D, the potential health properties of these seaweeds stands to generate significant interest in the marketplace and should be investigated concurrently. Extracts from seaweeds contain a complex mixtures of 100s of natural compounds, to which health benefits such as antioxidant, anti-cancer, anti-inflammatory, and immuno-stimulatory have been ascribed (Winberg et al. 2009). Seaweeds are particularly known to produce cytotoxic (cancer cell killing) compounds however further investigation is required to fully understand their mechanism of action in order to improve their properties, understand and reduce side effects, and determine the best form for delivery (diet or supplements). A standard method of determining the potential anticancer activity of a seaweed extract is to measure its cytotoxicity, i.e. its ability to kill cancer cells. However, this only reveals that a compound can kill cancer cells; it doesn't explain how it does so.

One way to determine if and how an extract can kill cancer cells is to measure inhibitors of kinase enzymes which are crucial for the survival of cancer cells. Kinase inhibitors provide a new target for anticancer agents that are more specific, efficacious and with less toxic side effects, and there are several examples already in clinical trials (Dancey and Sausville 2003). Kinases have also been implicated in a host of other diseases including atherosclerosis, autoimmune diseases, central nervous system disorders and Alzheimer's disease. A small number of kinase inhibitors have already been discovered in seaweeds, including styloquinonic acid from the brown alga *Stypodium zonale* (Wessels et al. 1999), cyloartanol sulfates from *Tydemania Expeditionis* (Govindan 2008), and sulfated triterpenoids from a green alga belonging to the *Tuomoya* genus (Clement 2003). Therefore screening for kinase inhibition properties of seaweed extracts selected in this project was selected as priority method for assessing potential anti-cancer properties.

**Table 3. Nutrient uptake efficiency as reported in other studies for different macroalgal genera in integrated aquaculture. Macroalgal uptake efficiency is measured as the average reduction (%) of nutrient concentration between influent and effluent waste waters.**

Culture facility	Cultured organisms	DIN (%)	NH <sub>4</sub> <sup>+</sup> (%)	SRP (%)	Source
Tank	seabream/ <i>Ulva</i>	34 - 49			(Neori et al. 1996)
Tank	salmon/ <i>Laminaria</i>	26 - 40			(Subandar et al. 1993)
Tank	seabream/ <i>Ulva</i>	19 - 97			(Jiminez del Rio et al. 1996)
Tank	shrimp /oyster/ <i>Gracilaria</i>	96			(Jones et al. 2001)
Tank	abalone/ <i>Gracilaria</i> ; <i>Ulva</i>	88		25	(Neori et al. 1998)
Pond	seabream/ <i>Ulva</i>		85 - 90		(Neori et al. 2003)
Tank	salmon/ <i>Gracilaria</i>		70 - 94		(Buschman et al. 1994)
Aquaria	milkfish/ <i>Kappaphycus</i> spp.		41 - 66		(Rodrigueza and Montano 2007)
Tank	seabream/ <i>Ulva</i>		40 - 56		(Cohen and Neori 1991)
Tank	seabream/ <i>Ulva</i>		17 - 39	9 - 21	(Krom et al. 1995)
Tank	salmon/ <i>Gracilaria</i>		90	32	(Buschman et al. 1996)
Tank	seabream/ <i>Ulva</i>		85		(Vandermeulen and Gordin 1990)
Tank	abalone/seabream/ <i>Ulva</i> , <i>Gracilaria</i>		80		(Neori et al. 2000)
Tank	clams/ <i>Hypnea</i>		70		(Langton et al. 1977)
Cage / channels	fish (unknown sp.)/ <i>Ulva reticulata</i>		65	33	(Msuya 2008)
Tank	sewage/oyster/ <i>Chondrus</i> ; <i>Ulva</i>				(Ryther et al. 1975)
Aquarium	fish/ <i>Gracilaria</i> ; <i>Ulva</i>				(Harlin 1978)
Tank	salmon/ <i>Gracilaria</i>				(Haglund and Pedersen 1993)
Cage culture	yellowtail/ <i>Ulva</i>				(Hirata and Kohirata 1993)
Open culture	oyster/ <i>Kappaphycus</i>				(Qian et al. 1996)
Pond/canal	shrimp/ <i>Gracilaria</i>				(Phang et al. 1996)
Cage culture	salmon/ <i>Gracilaria</i>			27	(Troell et al. 1997)
Pond/aquarium	milkfish/ <i>Gracilariopsis</i>				(Alacantara et al. 1999)
Cage culture	salmon/ <i>Porphyra</i>				(Chopin et al. 1999)
Tank	abalone/ <i>Palmaria</i>				(Evans and Langdon 2000)
Pond/ditch	shrimp/ <i>Gracilaria</i>				(Nelson et al. 2001)
Tank	Fish/ <i>Caulerpa</i> spp.				(Nicholas and de Nys 2008)
Ponds	Prawns/green macroalgae				(de Paula Silva et al. 2008)

# Objectives

The objectives of this research project were to set up tumble culture trials for a range of seaweed species native to the NSW south coast at both laboratory and pilot farm scales, in addition a selection of seaweed species was to be screened for anti-cancer activity. The objectives were achieved through four strategic milestones and can be summarised as follows:

## **Source and select seaweed species**

Sourcing and selecting the seaweed species that were abundant and available would provide the most immediate and reliable source of seaweeds to continue cultivation trial development. In addition, these would be the most suitable from an environmentally sustainable perspective.

## **Pilot cultivation trials in laboratory conditions**

Laboratory cultivation in artificially lit aquaria in natural seawater was used to determine if the different seaweed species could adapt to artificial tumble culture conditions easily. In addition the effect of light, nutrient uptake rates and sensitivity to four water treatment were tested experimentally.

## **Pilot commercial cultivation in IMTA**

The species of most marketable and reputable potential in IMTA systems were trialled in a commercial pilot scale IMTA system with fed marine fish.

## **Anti-cancer screening**

Our project aims to screen 12 temperate seaweed species collected from the Illawarra region for the presence of natural compounds displaying kinase inhibitory activity. The algal samples collected will be extracted, tested and their bioactive constituents purified and identified, potentially giving rise to a new class of anticancer agents.

# Methodology

## Source and select seaweed species

The physiological requirements, the biological cycles and propagation of different seaweed species can vary widely. In addition, only a few seaweeds will be considered suitable for integration into IMTA systems with high nutrient loads and tumble culture conditions. Therefore, the species considered here for cultivation and cancer screening trials were selected on the basis of some or all of the following criteria:

- abundant and native to the NSW south coast
- reported high nutrient stripping capacity from other studies
- reported elsewhere as cultivable, and in particular in integrated fish or shellfish culture systems
- medical, nutritional or other high value marketable product potential
- opportunistic species that developed within cultivation trials

Field trips for seaweed collection were undertaken throughout the year from June 2008 until June 2009 to cover the full seasonal cycle of life stages that many seaweeds exhibit. The spatial range of collection was from between Gerringong (34° 44' S, 150° 50' E) and Bawley Point (35° 30' S, 150° 23' E), on the NSW south coast of Australia. Abundant and interesting species in each of the red, green and brown seaweed groups were collected for pilot cultivation trials, nutritional and light experiments as well as screening for anti-cancer activity. Upon collection, samples were immediately placed in plastic bags of seawater in dark cool conditions in an Esky for transport to the laboratory.

## Pilot cultivation trials in laboratory conditions

### Culture maintenance trials

Over a twelve month period, 26 species of seaweeds were collected and trialled in tumble culture aquaria with artificially lit conditions. Nutrient doses were provided every 3 days to maintain nutrient levels at approximately 0.5mg/L of total ammonium nitrogen (TAN) to represent potentially high fish nutrient waste levels in an IMTA system. To determine which of the 26 species of seaweed could be maintained in artificial culture conditions easily in the laboratory, all of the 26 species of seaweeds were trialled in individual aquaria cultivation in the lab under a 12:12hr illumination cycle using Osram Bio-lux fluorescent lighting for aquarium plants. This selected lamp system reflected the sun's full spectrum, albeit at a lower intensity.

Cultures were maintained as tumble culture using air lines attached along the bases of aquaria. Flow was adjusted to suit the species in order to keep it just suspended and tumbling gently. Growth was determined by weighing seaweeds at weekly intervals after spinning for 1 minute in a salad spinner to remove excess water and condition by observation of the blades.

### Nutrient uptake trials

Macroalgae have been integrated with aquaculture with varying success. Different studies report that species of *Ulva* and *Gracilaria* are ideal candidates for integration with aquaculture. *Gracilaria chilensis* is capable of removing between 90-95% of ammonium from salmon farm effluent (Buschmann et al. 1996), whilst *Ulva rigida* is capable of stripping with high efficiency (more than 90%) the dissolved inorganic nitrogen from cultured gilthead seabream effluent (Jiménez del Río et al. 1996). However the data from the different integrated aquaculture studies listed at Table 2, indicate

that the range of dissolved inorganic nitrogen that can be removed (ammonium and nitrate), varies from 19% to 100% for ammonium, 17% to 90% for ammonium and nitrate, and 9% to 56% for phosphate. The biofiltration of phosphate and nitrate is less efficiently removed in many integrated cultivation systems. Buschmann et al. (1996) reported that *Gracilaria chilensis* only removed 32% of the phosphate and a similar low removal efficiency was reported by Neori et al. (1998) using *Gracilaria* and *Ulva* species.

Whilst there is no doubt that ammonium is being efficiently removed by *Ulva* and *Gracilaria* in integrated aquaculture (2000; Neori et al. 2003), few studies have investigated the efficient removal of nitrate and phosphate (except see Hernandez (2005)). Additionally, no large scale studies could be found that measured the macroalgal removal efficiency of nitrite and urea. It has been suggested in the literature that future studies must address the development of integrated systems to further reduce the outflow nutrient concentration, in particular phosphate and nitrate (Hernández et al. 2005). A promising solution may be the use of a more diverse range of species with different nutrient preferences, resulting in enhanced uptake of a wider range of nutrients such as has been suggested from ecological studies in rock pools (Bracken and Stachowicz 2006).

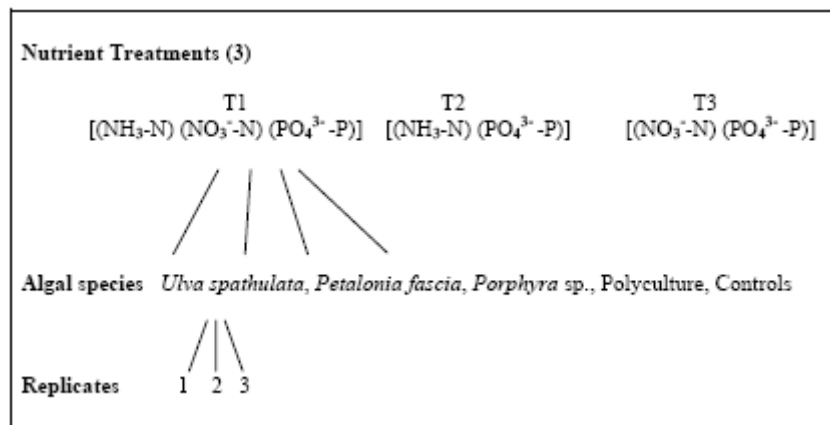
In this pilot study to determine if different species of seaweeds demonstrated different nutrient uptake rates and preferences, three seaweed species from each of the three major divisions (Rhodophyta: *Porphyra* spp., Phaeophyta: *Petalonia fascia* and Chlorophyta: *Ulva* sp.) were collected at low tide in June 2008 (Figure 1). These species were chosen as they each represented one of the three algal types of red, green and brown, have been identified as having high nutrient uptake rates, are of value as a nutritional crop and were growing together on the rocky shore indicating that they may be suitable for sharing nutrient resources efficiently in polyculture conditions. As the species all have very similar morphology (i.e. thin flat thalli), with high surface area to volume ratios (SA:V), it was expected that they might have similar rates of nutrient uptake but with different nutrient preferences (Hein et al. 1995).



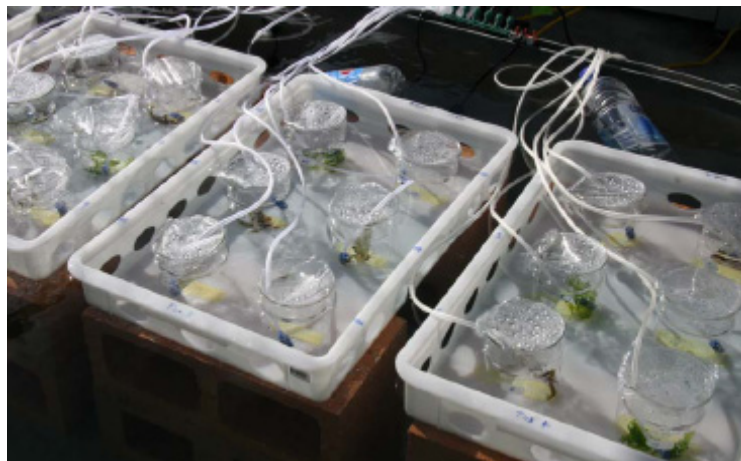
**Figure 1.** Three species of seaweed, *Ulva* sp. *Porphyra* sp. and *Petalonia* sp., co-occurring on the rocky intertidal shore at Werri Beach in southern NSW.

Three species of co-occurring, intertidal macroalgae were used for the experiment (Fig. 1). Approximately 10 plants each of *Ulva spathulata*, *Porphyra* sp. and *Petalonia fascia* were set up in

replicated (n=3) trials to test the different seaweed preference for nutrient sources, compared uptake rates and to see if the combination of nutrient forms available affected uptake rates (Fig. 2). Seaweeds were cultivated in monoculture and polyculture between the hours of 10am to 2pm to determine differences in uptake rates of total nutrients (Fig. 3).



**Figure 2. Experimental replication of nutrient uptake trials in different treatments of nutrient combinations.**



**Figure 3. Nutrient uptake experimental set up using a temperature controlled water bath and natural sunlight to test the nutrient uptake rates of three monocultures of seaweeds (*Ulva* sp., *Porphyra* sp. and *Petalonia* sp.) and one polyculture combination of all three types.**

## Light Effects Trials

Light is a critical factor for the growth and condition of algae and plants, with different species suited to different light intensities and, particularly in the case of submerged algae, different wavelengths. Here the growth of *Ulva* sp. in relation to light exposure was of interest in order to

- develop protocols for lab cultures for maintenance of stock cultures,
- determine how relevant lab culture experiments are in relation to interpretation for ambient light and temperature conditions
- to determine the effects of light conditions on the photosynthetic pigment composition in the algae
- to determine if greenhouse synthetic film designed for green plant culture is suitable for the culture of green macroalgae



Fresh *Ulva* sp. thalli were collected from Bannister Head in March 2009 and rinsed in clean filtered seawater. Healthy thalli with blades in good condition (no sporing, tissue damage or visible epiphytes) were selected and 18g was placed in 3 replicate 10L clear, plastic, cubic containers in each of three treatments: natural light, greenhouse shade and fluorescent “Lifelux” with the full spectrum of solar radiation. Life lights were selected as *Ulva* sp. are naturally exposed to the full spectrum of sunlight in their intertidal habitats.

Algal samples in the 9 containers were tumbled with air exiting from perforated plastic tubing attached to the base inside each container, and a plant nutrient fertilizer (Thrive) with seaweed extract was added every 3<sup>rd</sup> day to maintain ammonia nitrogen (NH<sub>3</sub>-N) (TAN) concentration of between 0.1-0.5mg/L. This concentration reflected the range of TAN in fish waste water from the pilot farm and. Cultures were maintained at 18°C in a heat pump controlled water bath for 10 days, and the algal condition (PAM photosynthetic yield) was measured on each of 5 days during the culture period (PAM settings: ML 7 Damp 3 Gain 3 SI 5 Wd 0.6).

On Day 10, samples were removed and frozen immediately and stored in a -80C freezer for analysis of chlorophyll a, b and carotenoid content. This was done according to standard spectrophotometry (Parsons and Strickland 1963; Jeffrey and Humphrey 1975; Parsons et al. 1984; Porra et al. 1989) and high pressure liquid chromatography (HPLC) (Wright et al. 1991) laboratory methods at the University of Wollongong. Between 1-10mg of algal tissue samples were prepared with liquid nitrogen and ground with acetone and sand in a mortar and pestle to extract pigments. Samples were added to Eppendorf tubes with NaHCO<sub>3</sub>, mixed and kept on ice in the dark for 20 minutes. Samples were centrifuged for 3 minutes and the supernatants used for analysis with a spectrophotometer for chlorophyll a, b and carotenoids using equations:

$$1) \text{ Chl } a = \frac{((12.25 \times (A_{664} - A_{750}) - 2.55 \times (A_{664} - A_{750}))) \times V \times D}{W}$$

$$2) \text{ Chl } b = \frac{((20.31 \times (A_{664} - A_{750}) - 4.91 \times (A_{664} - A_{750}))) \times V \times D}{W}$$

$$3) C_{(x+c)} = \frac{((((1000 \times (A_{470} - A_{750}))) \times V \times D))) - 1.82 \times C_a - 85.02 \times C_b}{W} 198^{-1}$$

where:  $C_a = \frac{((12.25 \times (A_{664} - A_{750}) - 2.79 \times (I_{647} - G_{750}))) \times V \times D}{W}$

$$C_b = \frac{((21.5 \times (A_{647} - A_{750}) - 5.1 \times (A_{664} - A_{750}))) \times V \times D}{W}$$

and

- Chl a = chlorophyll a (µg/L)
- Chl b = chlorophyll b (µg/L)
- C<sub>(x+c)</sub> = total carotenoids (µg/L)
- A<sub>x</sub> = absorbance spectrum
- V = volume of extract (mL)
- D = dilution
- W = fresh weight (g)

## Water/Aquaculture Treatment Effects

Three common methods of aquaculture water treatment for sterilisation or treatment of fish were trialled to test the suitability for sterilisation of seaweed or the tolerance of seaweed to common fish treatments. The treatments used were chlorine, formalin and freshwater treatment.

## Pilot commercial cultivation in IMTA

*Ulva* sp. was integrated into a pilot scale fish and seaweed IMTA system to determine the growth and yield as well as the suitability for integration as a nutrient biofilter with an Australian marine fish species.

## Anti-cancer screening

Twelve species of red, green and brown alga (Table 4) were collected by hand at low tide in the intertidal zone and down to 2m depth at various locations along the Illawarra coastline, in August and September, 2008.

**Table 4. Seaweed species collected and assayed for anti-kinase activity screening**

Entry	Phyla	Common Name	Scientific Name	Location
1	Rhodophyta	red algae	<i>Porphyra</i> sp. 1	Brawley Point
2	Chlorophyta	green algae	<i>Ulva</i> sp. (Enteromorpha form)	Bawley Point
3	Phaeophyta	brown algae	"Brown ribbon"	Bawley Point
4	Phaeophyta	brown algae	<i>Petalonia</i> sp.	Bawley Point
5	Chlorophyta	green algae	<i>Ulva latuca</i>	Bawley Point
6	Phaeophyta	brown algae	<i>Phyllospora comosa</i>	Bannister Head
7	Phaeophyta	brown algae	<i>Colopmenia</i> sp. 1	Bannister Head
8	Phaeophyta	brown algae	<i>Ecklonia radiata</i>	Bannister Head
9	Chlorophyta	green algae	<i>Codium</i> sp.	Bannister Head
10	Phaeophyta	brown algae	<i>Sargassum vestitum</i>	Bannister Head
11	Rhodophyta	red algae	<i>Porphyra</i> sp. 2	Gerringong
12	Chlorophyta	green algae	<i>Ulva</i> sp. (blade form)	Gerringong

For all twelve taxa, samples were prepared according to the procedure of Wright (1998) by homogenizing 8 g of either fresh or frozen material in 20 mL of non-denatured 100% ethanol using an Invitro IKA T10 basic Ultra-Turrax homogeniser, and the resulting suspension steeped overnight at 4 °C in a 20 mL scintillation vial. The resulting extract was filtered through a Whatman filter paper and the filtrate evaporated to dryness and weighed. A solution of the extract of known concentration of 5 mg/mL was prepared using non-denatured 100% ethanol. This solution was diluted 1:5 with distilled water and used directly in the following assays. The control samples were aqueous solutions of 20% ethanol. During the assay the sample is further diluted 1:10 with a buffer to give a final extract concentration of 100µg/mL.

Protein kinase A inhibitory activity was determined using the Kinase Glo<sup>®</sup> luminescent kinase assay according to the manufacturer's procedures (Promega 2007). All testing was conducted in white 96 well microlitre plates (Corning, Cat. #3912) and the reaction mixture is outlined in Table 2. After an incubation time of 30 minutes at room temperature, 50 µL of Kinase-Glo Reagent was then added to all wells and the reagents again allowed to incubate for another 30 minutes. Results were read on a RMG Labtech FLUOstar Optima<sup>®</sup> luminometer (Fig. 4).

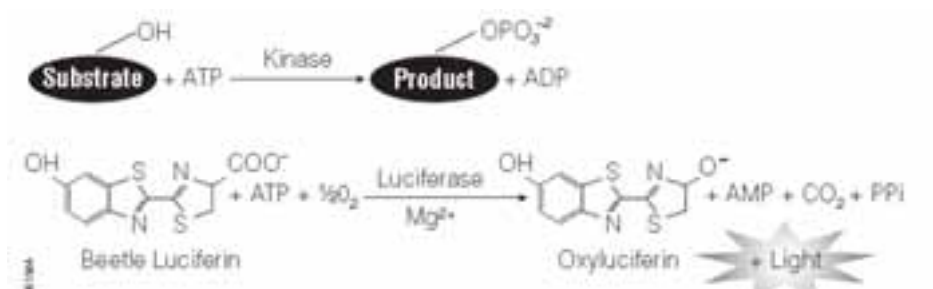





Figure 4. The Kinase Glo<sup>®</sup> Assay Reaction (Promega, 2007).

# Results

## Selected seaweed species

The following 18 seaweed taxa were collected in the field for tumble culture cultivation trials and anti-cancer screening.

Photo	Algal Species	Collection Site	Collection Date	Culture Trial	Anti-kinase assay
	<i>Ulva</i> sp.	Gerroa, Gerringong, Mollymook, Bawley Point	13/08/2008 - 30/05/2009	lab	assayed
	<i>Cladophora</i> sp.	Pilot farm	20/6/2009		
	<i>Bryopsis</i> sp.	Pilot farm	20/6/2009	pilot	



*Ceramium* sp.

Pilot farm

25/11/2008



*Filamentous reds*  
(potentially other life  
stage *Asparagopsis*  
*armata*?)

Pilot Farm and Lab

15/7/2009



*Gelidium* sp.

Jones Beach

3/09/2008

yes

collected



*Pterocladia*

31/5/2009



*Porphyra* sp. 1

Bawley Point

13/08/2008

yes

assayed



"Spaghetti" *Gracilaria*    Vincentia    5/11/2008    yes



*Gelatinous red*  
(maybe *Kappaphycus* sp.)    Pilot farm    31/5/2009    yes



*Gelatinous red*  
(maybe *Kappaphycus* sp.)    Pilot Farm



*Sargassum* sp. 2    Narrawallee    2/10/2008    yes    collected



*Sargassum* sp. 3    Ulladulla Harbour    5/11/2008    yes    collected



*Sargassum vestitum* Mollymook 05/11/2008 yes assayed



*Dictyota* sp. 1 Narrawallee 2/10/2008 yes collected



*Ecklonia radiata* Jones Beach 14/08/2008 assayed



*Petalonia* sp. Gerringong 13/8/2008 Assayed



Unknown brown sp. Narrawallee 2/10/2008 yes collected

## Pilot cultivation trials in laboratory conditions

### Culture maintenance trials

Of the 18 species of seaweeds collected, all of the red and green species were relatively easy to maintain for a up to 3 months with 12:12 hour light cycles and small regular additions of nutrients delivered from commercial fertilizers rich in ammonia (Thrive and Aquasol) (Fig. 5). Some demonstrated at least short term growth while others just seemed to main their size but in good condition. However all of the 6 brown seaweeds proved difficult to maintain, sporing shortly after introduction to aquaria and with rapid biomass disintegration within a few days.



**Figure 5. Tumble culture design in laboratory conditions.**

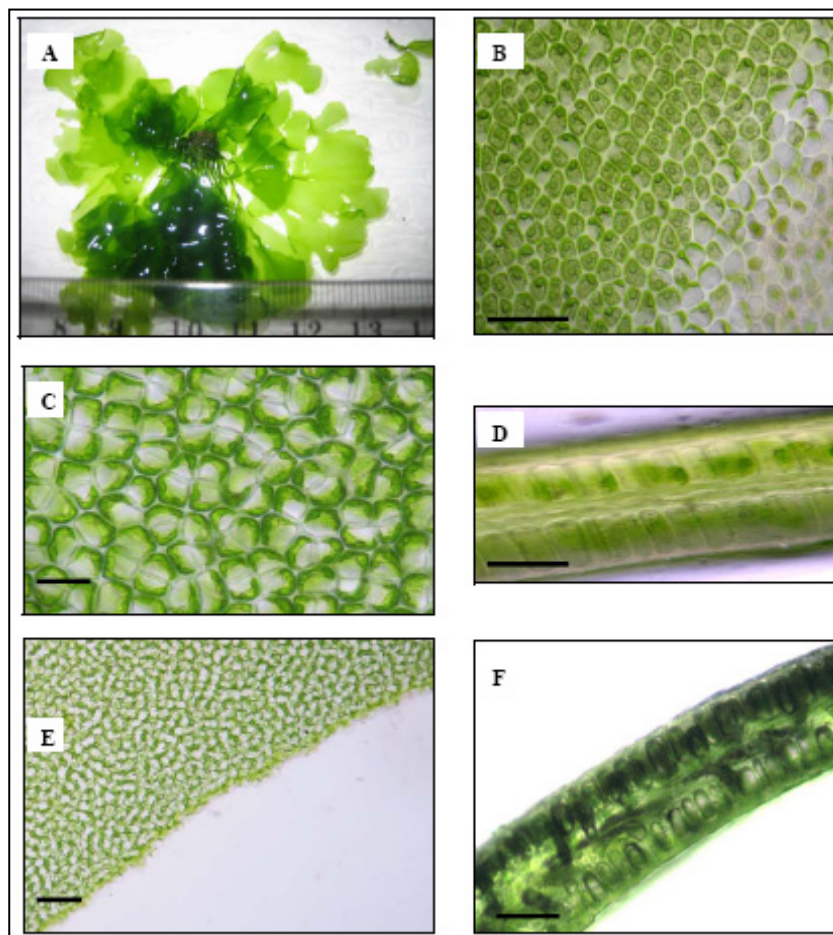
Performance of the green and red seaweed tumble culture trials are presented below, but as the brown seaweed pilot maintenance cultivation trials were not successful they are not presented here except for *Petalonia* sp. which was used for nutrient uptake trials. This does not mean that there is no potential for cultivation of brown species, but simply that the physiological requirements were not suitable. The primary cause of this might also have been that brown seaweeds are not suited to tumble culture as they are denser than the green and red species trialled and collect at the bottom of tanks. Brown seaweed sporling germination is widely practised in Asia and the potential to cultivate the sporling stage in tanks for values species should be investigated further (Chen 2004).

#### *Ulva* sp.

*Ulva* sp. were a key target for integration into an Integrated Multi-trophic Aquaculture (IMTA) system as research towards this type of cultivation has shown promising results, and some commercially viable *Ulva* culture systems exist internationally. As predicted, this species was relatively easy to maintain in laboratory aquaria culture conditions, however acclimatisation of the species is important and rapid changes in many of the physiological requirements (light and nutrients in particular) triggers sporing.

In addition, although it is an abundant and familiar sight on Australian coastlines, its nutritional value is underestimated for human consumption. It is a key source of nutrition for many herbivorous fish species and also for the famous marine iguanas (*Amblyrhynchus cristatus*) of the Galapagos Islands (Wilkelski and Wrege 2000). Similar nutritional value exists for human consumption and addresses limited minerals such as iron and calcium as well as operating as a functional food with preventative health potential for metabolic syndrome or early diabetes (Celikler et al. 2009). Earlier research has shown that *Ulva* consumption may help to lower cholesterol levels and improve gut health and that compounds within *Ulva* have been shown to have anti tumour, anti-influenza and anti-coagulant activities (Winberg et al., 2008; Lahaye and Robic, 2007).



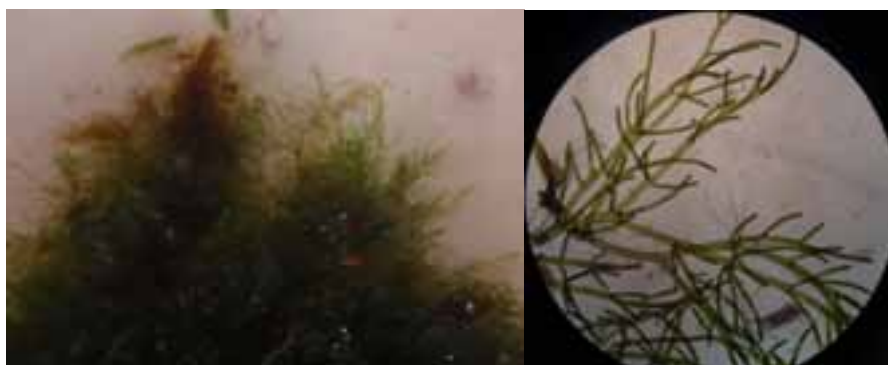


**Figure 6.** Excerpt Ullrich (2008). (A-F) *Ulva* sp. as (A) whole fresh plant (scale 1cm), (B) surface view of vegetative cells from marginal thallus region, reproductive cells on right of the photograph (scale 100 µm), (C) surface view of cells from mid thallus region (scale 20 µm), (D) cross-section of mid region of thallus (scale 50 µm), (E) Surface view of outer thallus margin s (scale = 100 µm), (F) cross-section of rhizoidal (lower) region of thallus (scale = 100 µm).

Due to the untapped nutritional benefits of *Ulva* and the well documented suitability for nutrient removal, this species was selected as the priority candidate for cultivation trials and in particular nutrient uptake studies in the lab (below) and scaled up to a pilot farm. Numerous *Ulva* species exist on the NSW coastline and although cellular morphology (Figure 6) can be used to distinguish some species, using morphology alone as a tool for identification of *Ulva* sp. is questionable (Woolcott and King 1999). Therefore all *Ulva* used in the following experiments is simply referred to as *Ulva* sp., and was sourced from the same location for each experiment.

#### *Cladophora* sp.

*Cladophora* was an opportunistic species (Fig. 7) that was seen in low numbers in cultivation aquaria. It is not thought to have much potential for valuable food or other products, and seems to be outcompeted by other green algae such as *Ulva* sp.



**Figure 7. Cladophora species found in low numbers as an opportunistic species in cultivation systems.**

*Bryopsis* sp.

This was an opportunistic species that developed in the high nutrient and high organic load culture systems (Fig. 8). *Bryopsis* is a coenocytic genus of the Caulerpales and is without cellular structure. Its growth rates and yields were dramatic but difficult to assess because of a very high water content and lack of cell structure. There is little evidence that it is suitable for consumption by anything other than mollusc species of the genus *Aplysia* (sea hares).

*Bryopsis* also contains many interesting compounds including kahalalide-F (USPTO Patent Application 20070117743) with antitumoral properties, and compounds as antiviral, antifungal agents and for the treatment of psoriasis. Additional interesting compounds include a lectin (Bryohealin (Yoon et al. 2008)) which is of use to a coenocytic algae such as *Bryopsis* in creating new cell membrane once cytoplasm is free in the water (Tatewaki and Nagata 1970).

Although it appears that this species is suitable as a nutrient stripping and high yielding green algae, its marketable properties are difficult to ascertain as it is poorly documented.



**Figure 8. Microscopy photos of *Bryopsis* sp. showing the coenocytic structure (no cells) and continuous cytoplasm.**

*Ceramium* sp.

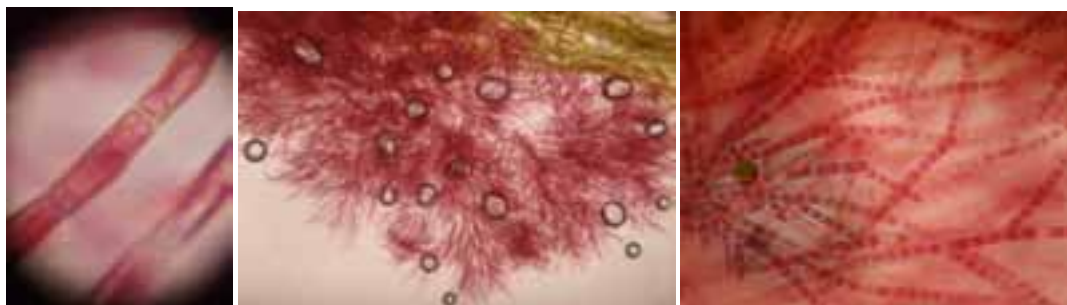
*Ceramium* sp. was an opportunistic taxa that established itself as an epiphyte on other cultivated seaweeds (Fig. 9). This is a well known epiphytic genus and has been reported elsewhere in IMTA seaweed cultivation systems, and it thought to have exceptionally fast nutrient uptake rates due to a large surface to volume ratio (Pedersen and Borum 1997; Schramm 1999) and can out compete other algae. However here *Ceraium* was present and notable, however it didn't reach competitive levels nor dramatically disturb the condition of seaweeds that it was attached to. This genus and the potential nutrient uptake benefits of it should be investigated further as it also has potential nutritional uses, in particular for abalone feed (Alcantara and Tadahide 2005).



**Figure 9. Microscopy photographs of *Ceramium* sp. that grew as an opportunistic epiphyte.**

#### Other filamentous reds

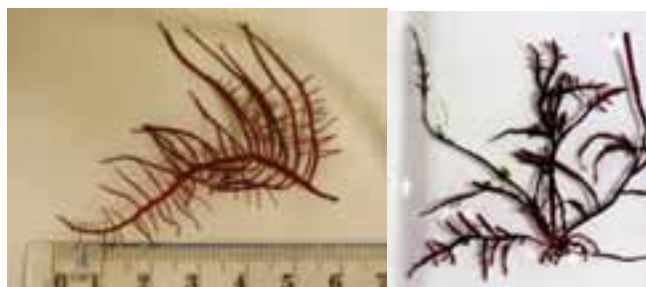
Many red algae have secondary life stages that have a filamentous morphology that is difficult to distinguish between species. Therefore this study groups such unidentifiable algae as opportunistic filamentous reds (Fig. 10). These deserve further investigation and identification. For example, one species with such life stage is *Asparagopsis armata*, and this species has been identified as a good candidate for cultivation in IMTA elsewhere (Luning 2004). It appears that the filamentous forms of red algae are particularly suited to tumble cultivation and are suited to higher nutrient environments, and therefore might be considered further for cultivation systems.



**Figure 10. Diverse filamentous red species that grew as opportunistically in cultivation trials.**

#### Gelidiaceae

Two species (Fig. 11) of the Gelidiaceae family were found and trialled as this family contains some of the most high quality agar with a high gel strength (Winberg et al. 2009). Although these species proved to maintain condition and health in artificial cultivation conditions, the growth rates were poor. Some further experimentation and light conditions could be trialled to determine if growth rates can be boosted, but otherwise it may be a case of being more suited to extensive sea based cultivation where it already is produced. The value of fresh product as a nutritional component of abalone diets should be considered however.

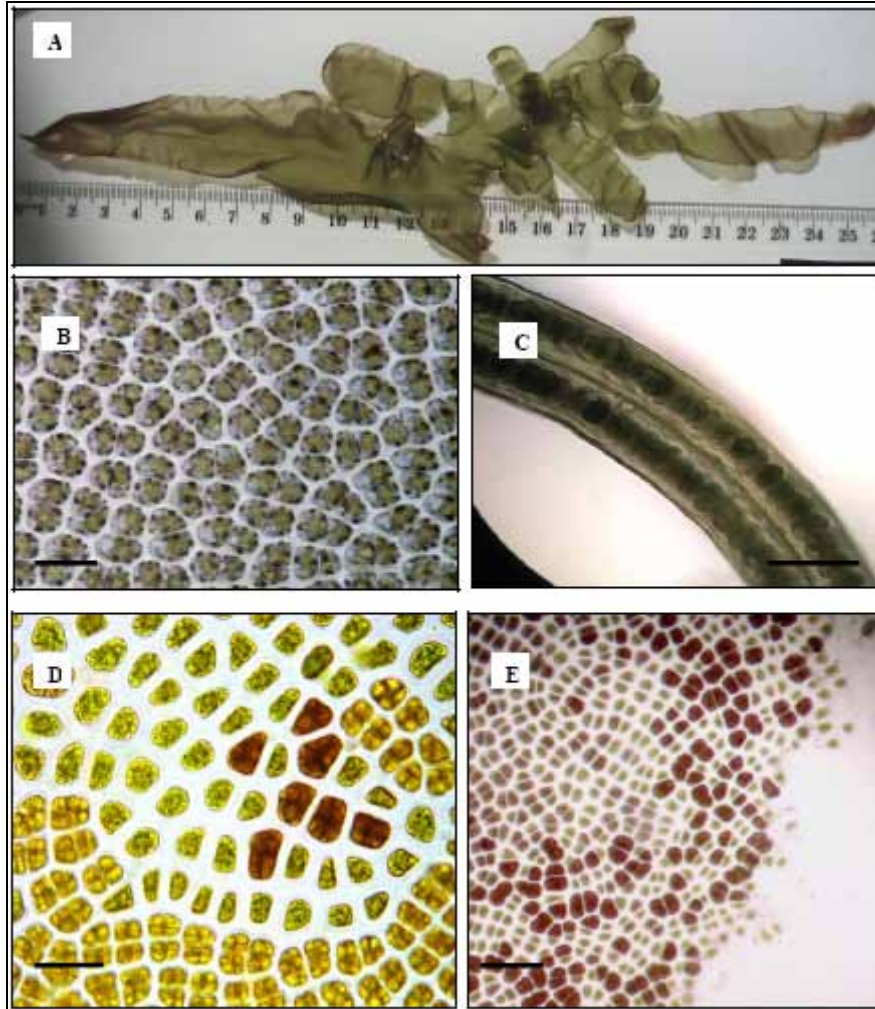


**Figure 11. *Gelidium* and *Pterocladia* spp. that maintained good condition but slow growth rates in aquaria cultivation trials.**



*Porphyra* sp.

*Porphyra* is notably difficult to identify by morphology alone even using cellular structure (Figure 12). However in this study, morphology was the only method available and it seemed as though there were two morphologically distinct species of *Porphyra* from different rocky shores on the south coast of NSW. Two species, *P. lucasii* and *P. columbina* are known to occur here (Edgar 1997) and possibly represent the two morphologies found (Figure 13).

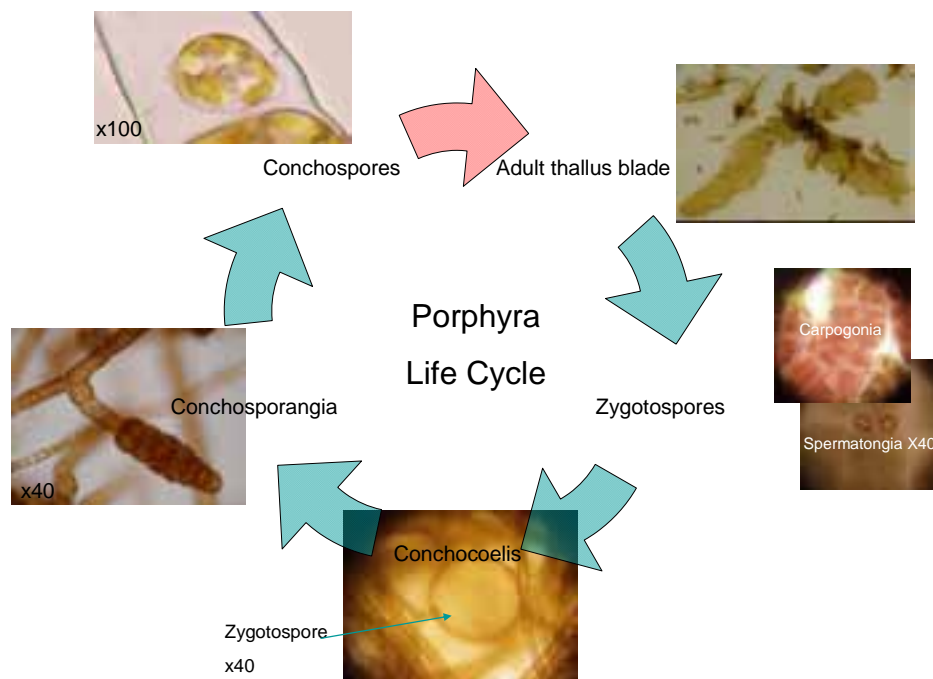


**Figure 12. (A-E) *Porphyra* sp. as (A) fresh plant (scale 1cm), (B) surface view of vegetative cells from mid thallus region (scale 20  $\mu$ m), (C) cross-section through mid thallus region (scale 50  $\mu$ m), (D) surface view of marginal region of thallus with reproductive cells (scale 50  $\mu$ m), (E) Surface view of marginal region of thallus showing irregular patches of reproductive tissue (scale = 100  $\mu$ m).**



**Figure 13. Two morphologically distinct species of *Porphyra* found on different rocky shores of the NSW south coast.**

*Porphyra* cultures were maintained successfully in indoor cultures for up to three months with constant 12:12 hour light conditions, however some wild collections started to spore at different times following collections days where indoor conditions differed in light and temperature. In some instances, preliminary trials were done to determine if the second life stage could be initiated, and this was successful to a degree with multiple conchocoelis stage forming (Fig. 14). Further trials need to be done to control the production of adult blades from chonchosporangia, as *Porphyra* is one of the higher value seaweed food markets. However the successful and currently abundant production in Asia needs to be taken in consideration for both international and domestic markets.



**Figure 14. Conchocoelis stage and reproductive structures of *Porphyra* sp. cultivated in the lab. Development of all lifecycle stages, except the redevelopment of adult thallus blades, was achieved.**

### Gelatinous reds

Three other gelatinous red seaweeds were found (Fig. 15) and trialled. Although good algal condition and health was maintained, growth rates were slow as above for the gelatinous reds of Gelidiaceae,.



**Figure 15. Gelatinous red species that had good culture maintenance characteristics but slow growth rates.**

### *Petalonia* sp.

*Petalonia* sp. (Fig. 16) was the one brown seaweed selected for nutrient uptake experiments as it grew in close proximity to *Ulva* sp. and *Porphyra* sp. in rocky intertidal habitat.

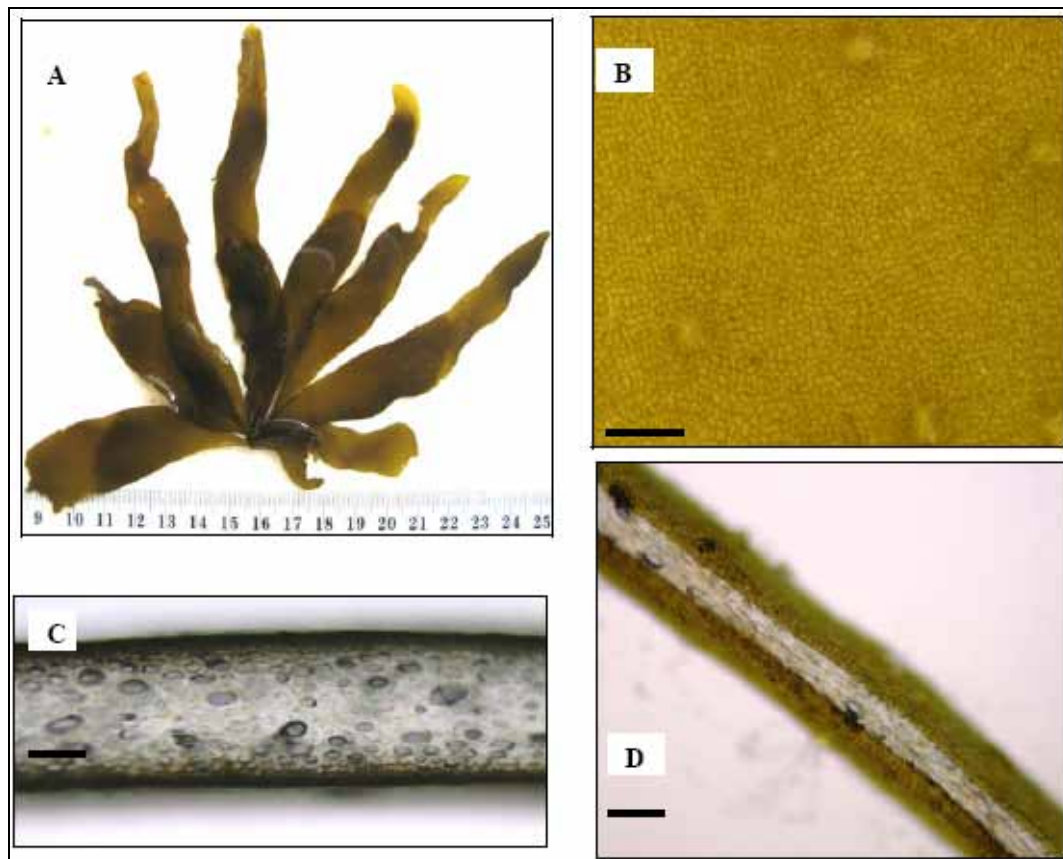
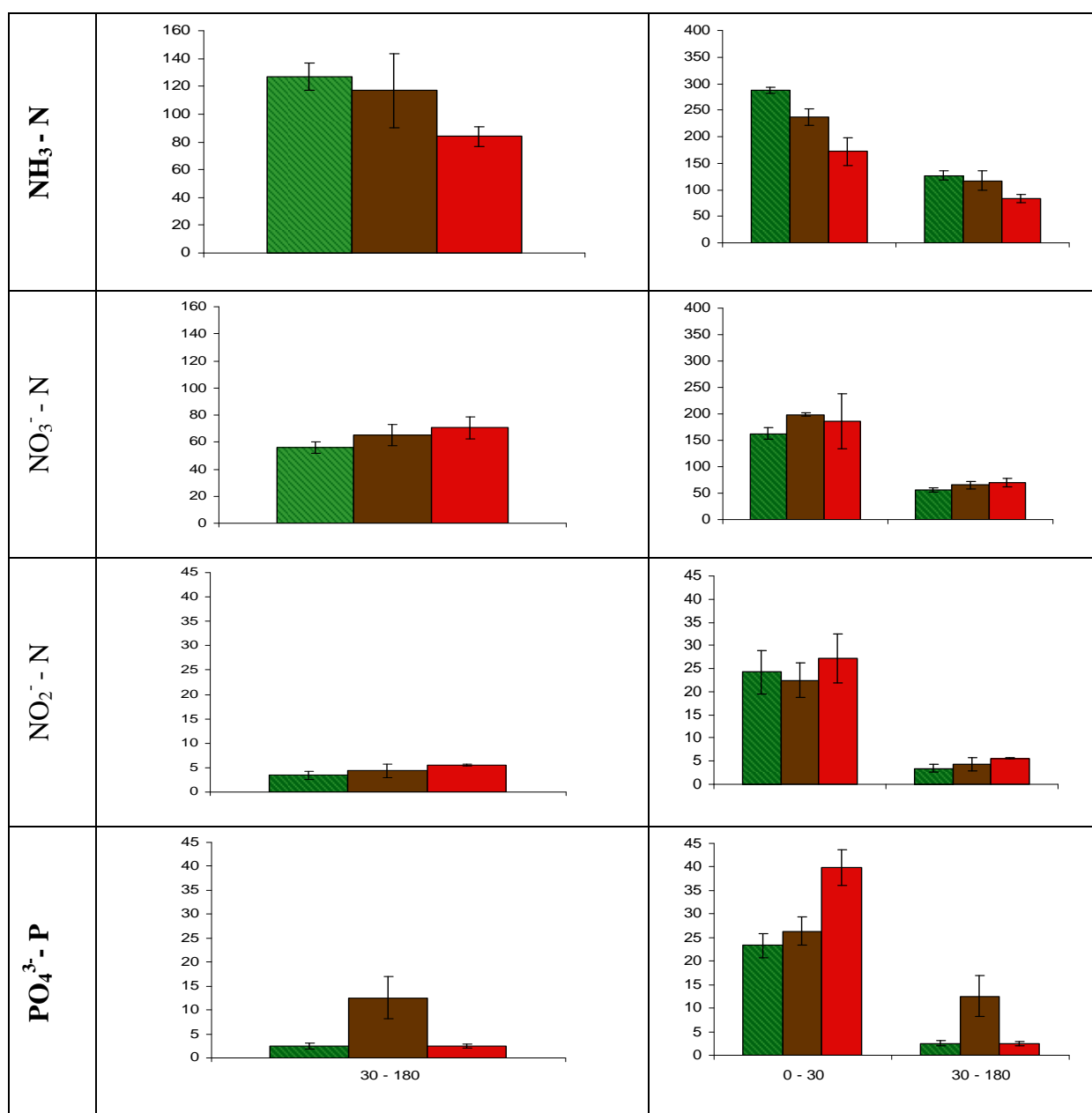


Figure 16. (A-D) *Petalonia fasciata* as (A) whole fresh plant, (scale 1cm), (B) surface view of mid region of thallus (scale = 100 µm), (C) cross-section through mid thallus (scale = 50 µm), (D) cross section through outer region of thallus (scale = 100 µm).

### Nutrient uptake trials

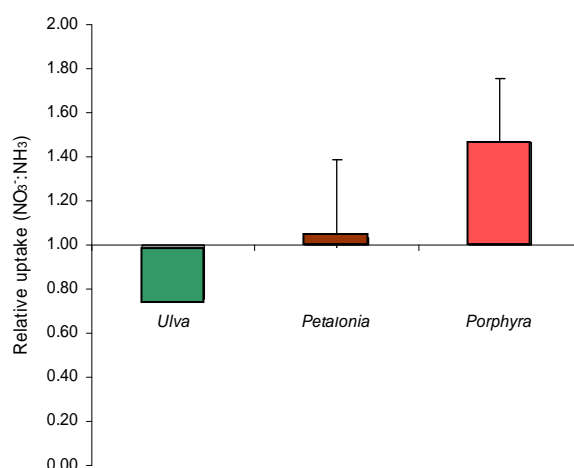
Full details of the nutrient uptake experiments are available in Ullrich (2008), but a summary of key findings are presented here. *Ulva* sp. showed a significantly higher uptake rate of ammonia nitrogen ( $\text{NH}_3\text{-N}$ ) than either of the other two species of algae (Fig. 17) during the initial (surge) uptake period, however over the course of the experiments this was only significantly greater than *Porphyra* sp. There was a trend of greater uptake rates of oxidised nitrogen forms (nitrate and nitrite) by *Porphyra* compared to the other species, but this was not significant. However, when combined, the relative uptake of nitrate versus ammonia (Fig. 18) was significantly different for *Ulva* and *Porphyra* spp., indicating that nutrient source partitioning may be occurring and provide for more efficient nutrient stripping in a polyculture system. This was however not demonstrated in subsequent experiments and needs to be investigated further.



**Figure 17. Nutrient uptake rates of ammonia, nitrate, nitrite and phosphate as  $V = \mu\text{mol}/(\text{g dry weight} \cdot \text{hour})$  for the three seaweed taxa *Ulva* sp. (green), *Petalonia* sp. (brown) and *Porphyra* (red). Uptake rates are given for the whole uptake trial period in the left hand column, and separated into surge and a saturated period rates in the right hand column.**

Uptake rates of phosphates ( $\text{PO}_4^{3-}$ ) was not significantly different between the seaweeds except for during the surge uptake where *Porphyra* sp. showed the highest uptake rates. These findings demonstrate good nutrient stripping potential for all three species, but no gains in nutrient uptake were demonstrated in a polyculture experiment.

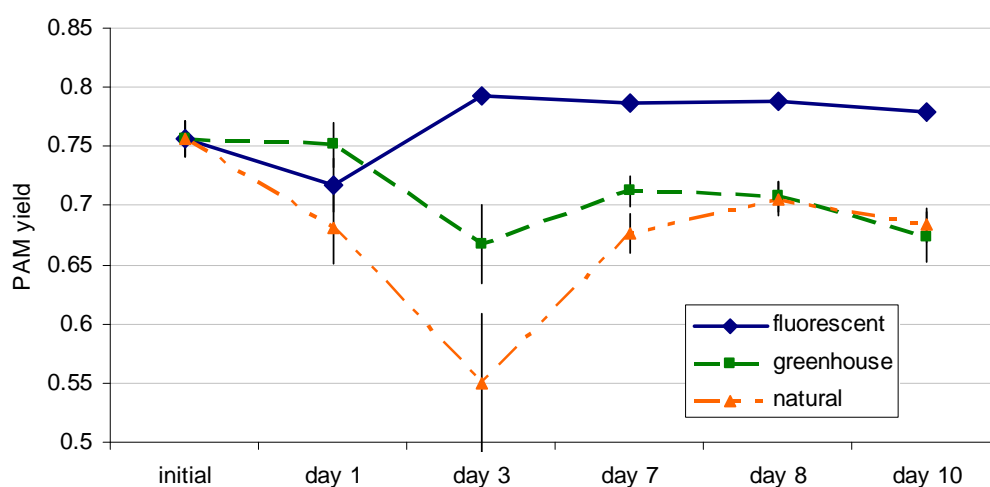




**Figure 18. The relative uptake preference of nitrogen sources for the three species of seaweed.**

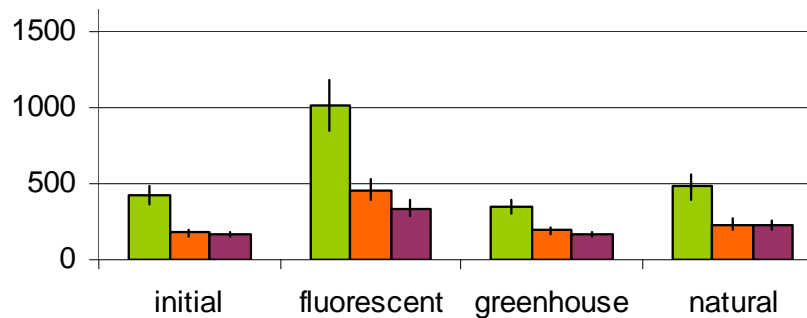
### Light effect trials

The PAM yield readings indicated a decline in condition of the seaweeds after initial establishment in culture vessels until day 3, when algae in fluorescent light conditions recovered quickly and PAM yield was elevated and maintained at above initial wild condition (Fig. 19). Algae in greenhouse or natural light conditions declined in photosynthetic yield until day 4 after which a partial recovery was made.

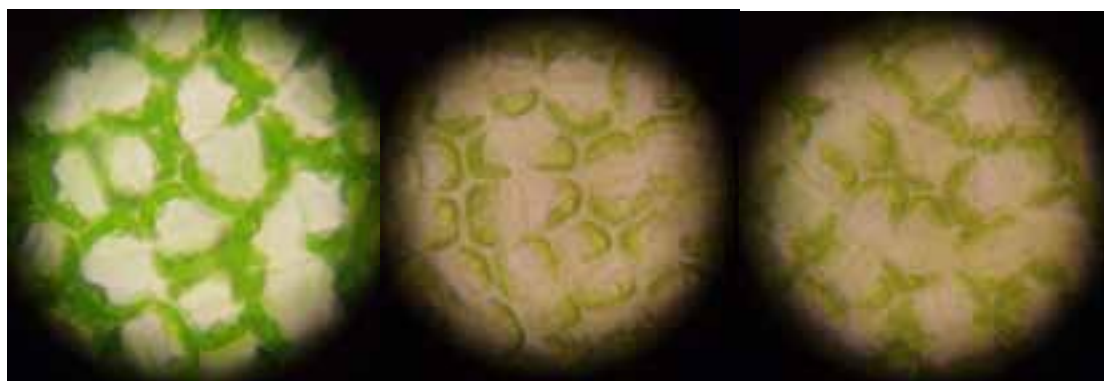


**Figure 19. PAM yields as a measure of condition of *Ulva* sp. samples in replicate (n=3) vessels in each of three culture treatments; fluorescent lights, ambient light under greenhouse film, in full ambient light conditions.**

The increase in PAM yield under fluorescent lights was reflected as a significant increase ( $>2.4$ ) in pigment levels for all chlorophylls (a and b) and total carotenoids (Fig. 20). *Ulva* sp. cultured under greenhouse and ambient light conditions and filtered seawater was comparable to wild collected *Ulva* sp. in natural conditions. This effect was also visible to the naked eye as a more intensive green colour of the thallus and blades, and as very thick and robust chloroplasts (Fig. 21).



**Figure 20.** Pigment levels (ug/mL) in *Ulva* sp. samples grown in experimental and replicated light conditions (initial, fluorescent, greenhouse and natural). Pigments measured include chlorophyll a (green), b (orange) and carotenoids (x+c) (red).



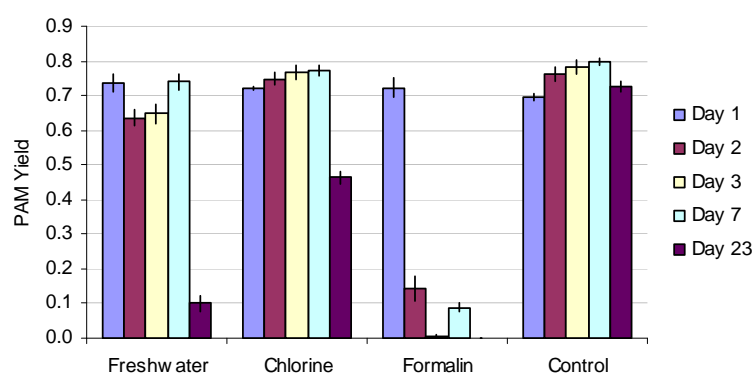
**Figure 21.** *Ulva* sp. at the end of the light condition culture experiment. *Ulva* from left to right grown under fluorescent light, greenhouse and natural light conditions for 10 days.

The initial decline in algal condition is typical of an acclimatisation period where initial tissue loss resulted in low or no net growth during the 10 day trials despite acclimatisation after day 4. *Ulva* sp. and other marine algae have previously been shown to require a period of acclimatisation to new culture conditions (Hatcher 1977).

Although the multifactor responses require further investigation to determine the relative influence of light, nutrients and other water quality parameters, it appears that culture under greenhouse conditions is comparable to natural ambient light, and in combination with elevated nutrients in the IMTA pilot provides algae with approximately a three fold increase in chlorophyll a, b and carotenoid levels. Fluorescent light and shade cloth also have an effect of increased pigments. The long term maintenance of *Ulva* sp. under low light or fluorescent light conditions remains to be seen, and may serve to provide a mother stock of seaweeds for grow out conditions.

## Sensitivity to aquaculture treatments

The exposure of *Ulva* sp. to concentrations of water sterilisation and pathogen removal treatments used in aquaculture systems demonstrated low tolerance to all treatments. Pure freshwater and chlorine exposure appeared to be tolerable for the first week; however the specimens deteriorated by day 23 whereas controls did not. Formalin toxicity to the seaweed was evident on the second day. Chlorine treatment may prove to be the most tolerable for *Ulva* sp. that is introduced to an IMTA system but only in low doses.



**Figure 22.** Algal health condition measured as light yield with Pulse Amplitude Modulated (PAM) fluorometer. Values between 7-8 indicate good photosynthetic performance with decreasing values indicating a loss of photosynthetic performance and therefore health/condition.

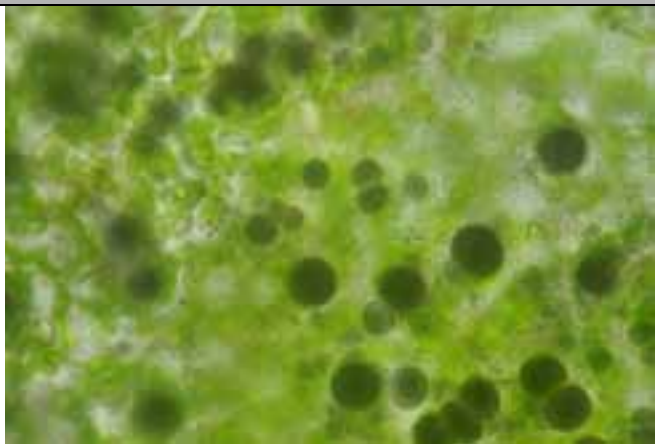





**Figure 23.** *Ulva* sp. thalli exposed to different water treatments; fresh water (UF1-3), formalin (UF4-6), chlorine (UF7-9) and normal seawater (control UN1-3).

## Potential Pests

A number of small algal or animal species were observed during cultivation trials that appeared to have the potential to become a pest species in cultivation. Not all of these species presented a noticeable challenge to cultivation and their presence was simply recorded, while others demonstrated challenges to cultivation.

**Table 5. Some algal and animal taxa that could prove to be potential pest species in seaweed cultivation systems.**

Algal and animal taxa that present potential problems as pests	
	Topical growth on <i>Ulva</i> sp. assemblages during low light conditions. This is not dissimilar, however still different in appearance to the reported “Brown Strangler” from South African IMTA farms with <i>Ulva</i> sp.
	Small zoo plankton and nematode fauna that may be grazing on seaweed thalli
	Filamentous red algae appeared in most seaweed cultures with time. Some of these may have been the juvenile stage of <i>Asparagopsis armata</i> , but many reds have similar filamentous life stages.
	Green microalgal species became abundant under low density and high nutrient condition of macroalgal culture. Diatoms were a problem with time in most cultures, and particularly during propagation experiments.

## Pilot commercial cultivation in IMTA

Scaling up of *Ulva* sp. into cultivation trials into an IMTA pilots system proved successful with good fish performance in relation to the seaweed cultivation module and recirculation. High protein and high quality *Ulva* production was achieved, although seasonal patterns in growth rate and consistency need to be addressed. High protein content and large blade morphology was achieved in the system as has been reported from studies elsewhere (Neori et al. 2004).



Figure 24. Seaweed cultivation at the pilot commercial IMTA system with fish.

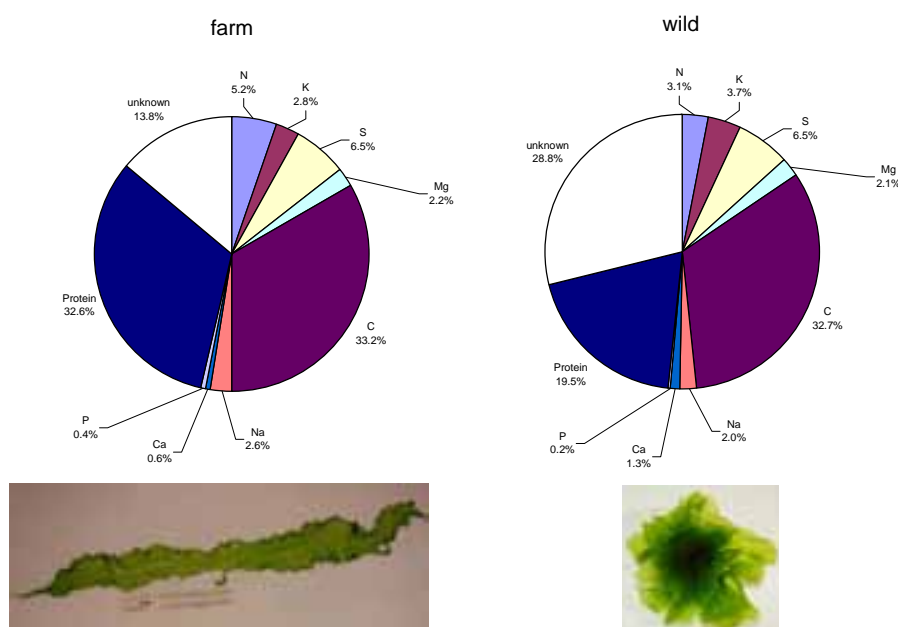


Figure 25. Tissue content as percentages of dried *Ulva* sp. cultured in elevated farm nutrient conditions versus natural/wild conditions.

## Anti-cancer screening

Of the 12 samples assayed, 9 (75%) exhibited a positive result in the first screen (Table 6). A repeat experiment, however, only produced positive results for four of the samples (i.e. a 33% successful hit rate). This is nonetheless a successful result as the probability of finding a “hit” in these types of natural product screens has generally been estimated to be as low as 0.7% (Barnes and Gallagher 2007). Three of the extracts, those from the red alga *Porphyra* sp. and the brown alga *Ecklonia radiata* and *Sargassum vestitum*, showed the highest levels of inhibition which were also consistent for both of the assay runs.

**Table 6. Inhibitory activities of extracts of 12 temperate SE Australian seaweed species towards protein kinase A. † nt = not tested, (-) 0% inhibition, (+) 1-25% inhibition, (++) 26-50% inhibition, (+++) 51-75% inhibition, (+++++) 76-100% inhibition**

Species	% Inhibition of PKA at 100 µg/mL <sup>†</sup>	
	Expt 1	Expt 2
<i>Ulva</i> sp. 2 (blade form cf. <i>australis</i> )	-	Nt
<i>Ulva</i> sp. 1 ( <i>Enteromorpha</i> )	+	nt
<i>Ulva</i> sp. (blade form cf. <i>lactuca</i> )	+	-
<i>Colopomenia</i> sp. 1	++	-
<i>Codium</i> sp.	+	-
<i>Porphyra</i> sp. 1	+++++	+++++
<i>Porphyra</i> sp. 2	-	nt
"Brown ribbon"	-	-
<i>Petalonia</i> sp.	++	-
<i>Phyllospora comosa</i>	+	+
<i>Ecklonia radiata</i>	+++++	+++++
<i>Sargassum vestitum</i>	+++++	+++++

This is consistent with previous findings (reviewed in Winberg (2009)) for brown seaweeds and *Porphyra* where in vitro cancer cell death has been shown. However here it is demonstrated how these seaweed extracts might kill cancer cells, through inhibition of kinase A; a potentially safe and targeted approach to cancer prevention and treatment.

The indication of high activity of anti-cancer properties in some of these Australian sourced seaweeds is consistent with the international reputation of seaweeds as important components of diets in populations that with low cancer rates (Yamori et al. 2001). It is of particular interest to note that our local native species of *Ecklonia radiata* might have comparable anti-cancer properties as the closely related *Undaria* sp., better known and globally marketed as Wakame. Two of the most potent extracts were from the brown alga *Ecklonia radiata* and *Sargassum vestitum*, showed 86% and 88% inhibition respectively (when tested at 100µg/mL). The red alga belonging to the *Porphyra* genus also showed excellent activity and as a well known food product deserve further investigation. The opportunity for these seaweeds to provide safe and effective application for preventative cancer treatment in the diet and/or as a medical application should be developed further.



# Implications and Recommendations

This report demonstrates that there are numerous seaweed species that hold potential for a vertically integrated seaweed industry in Australia. The project contributes to the early stages of progress towards realising a commercially viable seaweed cultivation industry and identifies some key challenges to be addressed in future R&D programs in order to achieve commercial reality.

Regarding the selection of the Australian seaweeds for cultivation, some promising species are presented here although many others remain to be tested. Here, species are regarded as promising depending on their potential for cultivation and/or demonstrated health benefits and high-value, marketable properties. In terms of cultivation technology development, it is recommended that further R&D focuses on refinement of consistent and high quality production of species that demonstrate good cultivation and nutrient stripping properties, such as *Ulva* and *Porphyra* sp.

Further R&D towards commercial cultivation should include gaining a better understanding and control of the biological lifecycles of selected seaweeds, including the physiological requirements and protocols for both propagation and grow-out conditions. Learning from industry and research organisations overseas is strongly recommended as there is a long history in many Asian countries in particular, but also elsewhere. This technology must then be adapted, through research and development, to the local species and cultivation conditions while considering opportunities for vertical integration with new and existing industries and markets in Australia.

Some species that proved challenging in tumble culture conditions, particularly brown sp. still deserve further cultivation technology research and development. Their potential health benefits could provide for substantially greater value, even considering more complex or sea based cultivation systems. For example, assays here indicated strong anti-cancer activity in some brown seaweed extracts. The application of these potential health benefits to provide safe and effective preventative cancer applications in the diet and/or supplement should be developed further. Similarly, gelatinous red seaweeds have other marketable qualities and are of particular interest as a feed in the abalone industry.

There are also genetic considerations for future research programs, particularly as species distinction within the same genus is difficult based on morphology alone for a number of taxa. For example the genus *Porphyra* showed two morphologically similar species with exceptionally different anti-cancer activity. The genetic identification of the species as well as the reasons for genetic expression of anti-cancer properties should be established through genetic research. Similarly, the genus *Ulva* has been shown to have strong species and strain diversity within the same morphology and habitat. High value products require isolation of reliable cultivars with desirable traits, thus again genetic determination of species and selection for genetic traits is required.

One immediate opportunity for cultivation that was demonstrated in this study is to integrate further seaweed cultivation trials and pilot systems with existing aquaculture enterprises where existing infrastructure and resources can be utilized. In addition, integration can serve to develop bioremediation technology for the nutrient rich waste from aquaculture facilities. Companies in existing aquaculture enterprises should be supported in trying to adopt and scale up the technology of seaweed cultivation, particularly for land based facilities where efficiency and environmental gains are important. Scaling up from lab cultures always brings new experiences and labour intensive and sometime costly barriers. This should also be undertaken concurrently to the development of Australian products, setting up systems to ensure compliance with food safety standards, and the development of markets.

Although seaweed cultivation and applications hold great potential, the technology in Australia is truly in its infancy and requires strong and strategic R&D support to achieve relevant and financially viable systems to attract industry investment. These concepts and recommendations should be pursued and

supported through R&D bodies and research organisations in collaboration with existing aquaculture industries that appreciate the opportunity for seaweed cultivation development. Following successful development of cultivation technology and marketable high value products for priority seaweeds, long term future applications in standalone seaweed cultivation systems, drought and salt affected areas and biofuels can be addressed.



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# Seaweed Cultivation Pilot Trials

— *Towards culture systems and marketable products* —

by Dr Pia Winberg, Dr Danielle Skropeta & Ms Alex Ullrich

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Globally, seaweed is the largest aquaculture production by volume at over eight million wet metric tonnes per annum (FAO 2003). Mostly this production is for traditional foods in Asia and the commodity markets of agar, alginates and carageenans. However, there is also untapped potential in smaller, high product value markets for nutritional and health applications. This is where Australia's best investment in a seaweed industry may lie.

This report presents findings that demonstrate an untapped potential for cultivation of a number of local Australian seaweed species, but it also identifies the challenges facing commercial-scale production. Importantly, it also provides evidence that Australia has the capacity and potential to undertake cutting

edge screening and development of healthy seaweed products, in particular, products with nutraceutical and anti-cancer applications.

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